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FWPCA METHOD FOR CHLORINATED HYDRO-CARBON PESTICIDES IN WATER AND WASTEWATER

Federal Water Pollution Control Administration
Cincinnati, Ohio

April 1969

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Federal Water Pollution Control Administration
Division of Water Quality Research
Analytical Quality Control Laboratory
Cincinnati, Ohio

CLEAN WATER

FWPCA METHOD FOR CHLORINATED HYDROCARBON PESTICIDES IN WATER AND WASTEWATER

April 1969

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FWPCA METHOD

FOR CHLORINATED HYDROCARBON PESTICIDES

IN

WATER AND WASTEWATER

PREFACE

The use of pesticides has become a routine practice in modern agriculture. While these compounds have great advantages in the control of predatory insects, they represent a possible danger to the aquatic environment when present in even trace concentrations.

The National Technical Advisory Committee on Water Quality Criteria has recommended "that environmental levels . . . not be permitted to rise above 50 nanograms/l." Many of the states have incorporated pesticide criteria in their water quality standards. Therefore, the monitoring of surface waters for pesticides is an essential part of our measurement of water quality.

The Analytical Quality Control Laboratory, assisted by FWPCA scientists experienced in the determination of pesticides, has prepared the following method for chlorinated hydrocarbon pesticides. In the opinion of the AQC Laboratory and its advisors, this method is the best available procedure at this time.

Because methods development and selection is a dynamic process, requiring continual efforts toward improvement, comments on the application of the method and suggestions for improvement are solicited from the analysts in the field. These comments should be addressed to:

Director, Analytical Quality Control Laboratory
Federal Water Pollution Control Administration
U.S. Department of the Interior
1014 Broadway
Cincinnati, Ohio 45202

The method has been adopted as the FWPCA Method for Chlorinated Hydrocarbon Pesticides and should be used by all Administration laboratories in acquiring data on the concentration of these materials in waters and wastewaters sampled by FWPCA.

David G. Stephan
Acting Assistant Commissioner
for Research and Development
Federal Water Pollution Control
Administration

CHLORINATED HYDROCARBON PESTICIDES
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Introduction

The science of analytical chemistry has advanced greatly in recent years. New methods yielding greater efficiency, selectivity, and sensitivity are constantly being developed. In keeping with this advance, the analytical chemist now has the means to measure minute quantities of pesticides, either singly or in combination. Laboratories capable of this measurement are now commonplace, providing pesticide pollution data on every major stream in the nation.

With the current and increasing sophistication of data storage and retrieval systems and the ever expanding toxicological and ecological information on the impact of pesticides, data obtained for several laboratories may be used in the combined assessment of pollution in a river system or in the nation. With such expanded use of data, the need for minimizing procedural error within each laboratory and optimizing analytical agreement between laboratories is paramount. The best means of obtaining agreement and insuring the validity of results from several laboratories is through the use of a single uniform method.

The Federal Water Pollution Control Administration's "Method for Chlorinated Hydrocarbon Pesticides in Water and Wastewater" will provide, if rigidly followed, the means of obtaining such agreement and validity.

The method offers several analytical alternatives, dependent on the analyst's assessment of the nature and extent of interferences and the complexity of the pesticide mixtures found. It is recommended for use only by experienced residue chemists or under the close supervision of such qualified persons. Specifically, the procedure describes the use of an effective co-solvent for efficient sample extraction; provides, through use of thin-layer and column chromatography, methods for the elimination of non-pesticide interferences, and the pre-separation of pesticide mixtures. Identification is made by selective gas chromatographic separations through the use of two unlike columns. Measurement is accomplished either by electron capture or microcoulometric detection. Findings are reported in nanograms per liter without correction for recovery data; but, such data is to be included in the report.

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FWPCA Committee on Methods Validation and
Analytical Quality Control

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Pacific Northwest Water Laboratory
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CHLORINATED HYDROCARBON PESTICIDES
in
WATER AND WASTEWATER

1. Scope and Application

1.1 This method covers preparation of the sample and the determination of the concentration of various chlorinated hydrocarbon pesticides, including some pesticidal degradation products and related compounds, by gas chromatography in water and wastewater.

1.2 The following compounds may be determined individually by this method: BHC, lindane, heptachlor, aldrin, heptachlor epoxide, dieldrin, endrin, Perthane, DDE, TDE, DDT, methoxychlor, endosulphan, γ-chlordane, and Sulfenone. Under ideal circumstances, Strobane, toxaphene, Kelthane, chlordane (tech.) and others may also be determined.

1.3 Various combinations (mixtures) of these compounds in a single sample may not be adequately distinguished, thereby causing high, low, or confused results through misidentification and/or one compound obscuring another of lesser concentration. Provisions incorporated in this method will minimize the occurrence of such interferences.

2. Definitions

2.1 Chlorinated hydrocarbons, for the purposes of this method, are defined as pesticides composed of carbon, hydrogen and chlorine.

2.2 Related compounds are halogenated materials which may also contain oxygen, sulfur, phosphorus or nitrogen, yet may be analyzed by this method.

3. Interferences

3.1 Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of gas chromatograms. All of these materials must be demonstrated to be free from interference under the conditions of the analysis. Specific selection of reagents and purification of solvents by distillation in all-glass systems is required. Glassware should be cleaned by rinsing successively with chromate cleaning solution, tap water, distilled water and finally with redistilled acetone. If the type and size of glassware permits, it is heated in a muffle furnace to about 400 C for 15 to 30 minutes. Plastics, except Teflon, can be the source of serious interference and must be avoided. For this reason, samples are collected in glass or Teflon bottles equipped with Teflon lined caps.

3.2 Sample treatment required to remove non-pesticide materials which cause interference may result in the loss of certain chlorinated hydrocarbons. It is beyond the scope of this method to describe procedures for overcoming all of the possible interferences that may be encountered, particularly with highly contaminated water and wastewater. Methods for eliminating or minimizing interferences are described in the sections on Clean-up and Separation Procedures (6.1 and 6.2).

4. Instrumentation

4.1 Gas Chromatography

The gas chromatographic system employed must be demonstrated to be suitable for the determination of chlorinated hydrocarbons with a

minimum of decomposition and loss of compounds of interest. The analyst must evaluate the individual system to demonstrate such capability. Column packings and column conditioning parameters as well as operation suggestions are included under Determinative Steps (8.).

4.2 Gas Chromatographic Detectors

4.2.1 Dohrmann Microcoulometric (MC) - The Dohrmann microcoulometric titrimetric detection system is the most specific detector for chlorinated hydrocarbons in general use. This titration cell (T-300-S) detector is capable of responding to low nanogram quantities for most of these materials under oxidative conditions, while almost entirely insensitive to other organics. Under optimum conditions it is capable of measuring 5 to 20 nanograms in a reproducible manner, depending on the chlorine content of the individual compound and its chromatographic qualities. However, relatively large samples must be processed in order to approach the sensitivity of the electron capture detector described below.

4.2.2 Electron Capture Detector (EC) - The electron capture detector is capable of responding to picogram quantities of chlorinated hydrocarbons, but because of detector volume limitations and interferences, the practical sensitivity is only 30 to 50 fold that of microcoulometry. Further, the relative sensitivity of one compound to another can vary greatly. As mentioned earlier (5.), the sample, and also in some cases, the solvents, reagents, glassware, and other hardware used for sample processing yield organics which greatly confuse

results obtained by electron affinity. Methods for minimizing the presence of these artifacts are covered in (3. and 6.).

The analyst must thoroughly acquaint himself with the description and theoretical information on these detectors. Review of technical papers and manufacturers' instructions on the specific detector employed are necessary to become familiar with its use and limitations.

4.3 Equipment and Sources

4.3.1 Detectors

a) Microcoulometric (T-300-S)

Dohrmann Instruments Company
1062 Linda Vista Avenue
Mountain View, California 94040

b) Electron Capture - Radioactive Source

(tritium or nickel 63)

(i) concentric tube design

Varian-Aerograph
2700 Mitchell Drive
Walnut Creek, California 94598

(ii) Parallel Plate design

This type of detector is supplied by many of the gas chromatograph manufacturers.

c) Electron Capture - Non-Radioactive Source

Beckman Instruments, Incorporated
2500 Harbor Boulevard
Fullerton, California 93632

4.3.2 Recorder - 1 millivolt, 1 sec., fullscale potentiometric strip-chart.

This type of recorder is supplied through many instrument manufacturers.

4.3.3 Kuderna-Danish Glassware (24/40 joints)

Snyder Column - three ball

Evaporative Flasks - 125 ml, 250 ml, and 500 ml

Receiver Ampoules - 2 ml and 5 ml

Ampoule Caps

Dohrmann Instruments
1062 Linda Vista Avenue
Mountain View, California 94040

4.3.4 Column Chromatography - Pyrex column (I.D. 19 mm, length 400 mm) with coarse fritted plate on bottom and Teflon stopcock, 250 ml reservoir bulb at top of column with flared out funnel shape at top of bulb--a special order.

Kontes Glass Company
Vineland, New Jersey 08360

4.3.5 Micro Syringes - (1, 10, 25, 50, and 100 µl)

Hamilton Company
Post Office Box 307
Whittier, California 90608

4.3.6 Separatory Funnels - two liter and four liter funnels with Teflon stopcock.

Pyrex or Kimble supplied through many distributors.

4.3.7 Thin Layer, Applicator and accessories, including UV source

Applied Science Laboratories
Post Office Box 440
State College, Pennsylvania 16501

Brinkmann Instruments, Incorporated
Cantiague Road
Westbury, New York 11590

also - from many other suppliers

4.4 Standards, Reagents and Solvents

4.4.1 Pesticide standards - highest available purity

City Chemical Company
132 West 22nd Street
New York, New York 10011

Applied Science Laboratories, Incorporated
Post Office Box 440
State College, Pennsylvania 16501

also - from the manufacturer

4.4.2 Florisil (60/100 mesh) - purchased activated at 1200 F and stored at 130 C.

Floridin Company
2 Gateway Center
Pittsburgh, Pennsylvania 15222

4.4.3 Sodium sulfate (A.C.S.) - granular, anhydrous

4.4.4 Pyrex wool - filtering grade

4.4.5 Solvents - hexane, diethyl ether, acetone, benzene, xylene, carbon tetrachloride - high purity, distilled in glass for pesticide analyses--either Nanograde type or purified in lab.

Burdick and Jackson, Incorporated
1953 South Harvey Street
Muskegon, Michigan 49442

Mallinckrodt Chemical Works
2nd and Mallinckrodt Streets
St. Louis, Missouri 63160

Matheson Coleman and Bell
Post Office Box 85
East Rutherford, New Jersey 07073

4.4.6 Gas Chromatographic Column Materials

Gas-Chrom Q (60-80 mesh)

Glass Wool (silanized with dimethyldichlorosilane)

OV-17

DC-200 (12,500 centistokes)

QF-1 (FS-1265)

Tubing (Pyrex or aluminum, 1/8 in. and/or 1/4 in. O.D.)

Applied Science Laboratories, Incorporated
Post Office Box 440
State College, Pennsylvania 16501

4.4.7 Silica gel-G with gypsum binder (No. 8076).

Warner-Chilcott Laboratories
Instruments Division
200 South Garrard Boulevard
Richmond, California 94801

5. Sample Extraction

The size of sample taken for extraction is dependent on the detection system employed and the sensitivity required for the purpose at hand. Background information on the pesticide levels detected in the past will help to determine the sample size required.

5.1 Microcoulometric Detection (Minimum detectable limit 5-20 ng/l)

Three liters of sample are placed into a 4-liter separatory funnel, equipped with a Teflon stopcock, and extracted with 150 ml of 15% ethyl ether in hexane by shaking vigorously for two minutes. The sample container is rinsed with each aliquot of extracting solvent prior to extraction of the sample.

5.1.1 The mixed solvent is allowed to separate from the water and this water is drawn off into a second 4-liter separatory funnel. The organic layer is passed through a small column of anhydrous sodium sulfate topped with a pledget of cotton (previously rinsed with hexane) and collected in a 500 ml tall form beaker. The extraction is repeated and the solvent treated as above. Approximately 100 ml of sodium sulfate saturated water is then added to the sample and a third extraction is

completed with 150 ml of hexane (not hexane-ethyl ether). This solvent too, after separation, is passed through the column of sodium sulfate. The column is then rinsed with several small portions of hexane and the solvent is blown out and recovered in the collection beaker containing the combined extracts. The contents of the beaker are partially evaporated to about 40 ml in a water bath at 70 C applying no air or vacuum.

5.1.2 If the sample is composed of relatively clean water, (eg. the upper Columbia River), apparently free from oils, coloring matter and other organics (ie., clear, near colorless, very fluid), the extract may be concentrated by transferring the partially evaporated sample extract with small portions of hexane to a 125 ml Kuderna-Danish (K-D) evaporative flask equipped with a 2 ml receiver ampoule. A three ball Snyder column is mounted to the top of the flask and the solvent is evaporated in a steam bath. After no more solvent actively distills the assembled K-D is removed from the bath and allowed to cool slightly. The Snyder column and the flask while still warm are rinsed with a small portion of hexane into the receiver ampoule.

The ampoule is disconnected and the concentrated extract is treated as described under Determinative Steps (8). For qualitative corroboration of the results, such extracts should subsequently be subjected to the clean-up and Separation Procedures (6.) and be re-examined by gas chromatography.

5.1.3 If the concentrated extract appears "dirty" (i.e. dark in color, cloudy, viscous) or is believed to contain materials which may interfere with measurement, proceed as described under Clean-up and

Separation Procedures (6.).

5.2 Electron Capture Detection (Minimum detectable limit $\leq$1 ng/l).

One liter of sample is drained into a 2-liter separatory funnel, equipped with a Teflon stopcock, and extracted with 60 ml of 15% ethyl ether in hexane, by shaking vigorously for two minutes. The sample container is rinsed with each aliquot of extracting solvent prior to extraction of the sample.

5.2.1 The mixed solvent is allowed to separate from the water and this water is drawn into a second 2-liter separatory funnel. The organic layer is passed through a small column of anhydrous sodium sulfate topped with a pledget of cotton (previously rinsed with hexane) and collected in a 250 ml beaker. The extraction is repeated and the solvent treated as above. Approximately 35 ml of sodium sulfate saturated water is then added to the sample and a third extraction is completed with 60 ml of hexane (not hexane-ethyl ether). This solvent too is passed through the sulfate column and collected in the beaker. The column is rinsed with several small portions of hexane and this solvent is blown out and recovered in the collection beaker containing the combined extracts. With a clean sample, the extract may be treated as described in (5.1.2 and 8.). With all other samples proceed as described under (6.).

6. Clean-up and Separation Procedures

A sample which may contain interferences (5.1.3) such as oil or wax like materials and other organics may be treated in several ways:

Column adsorption chromatography (6.1), thin layer chromatography (6.2) or column adsorption chromatography followed by thin layer chromatography. These procedures not only remove many of the oily or waxy substances which prevent distinct and specific gas chromatograms, but pre-separate mixtures of pesticides, thereby aiding the analyst in interpretation of subsequent gas chromatograms.

6.1 Florisil Column Adsorption Chromatography

The sample extract is concentrated to approximately 40 ml in a water bath at 70 C without air or vacuum. A 15 g charge of Florisil (4.4.2), which has been stored in an air-tight container at 130 C, is placed in a column (4.3.4) over a small layer (one-half inch) of anhydrous granular sodium sulfate. After tapping the Florisil into the column about a three-fourths inch layer of granular sodium sulfate is added to the top. The column, after cooling, is pre-eluted with about 75 ml of hexane. The pre-eluate is discarded, and just prior to exposure of the sulfate layer to air, the sample extract is quantitatively transferred into the column by decantation and subsequent hexane washings. The elution rate is adjusted to about 5 ml per minute with two eluates collected separately in 500 ml K-D apparatus equipped with 2 ml ampoules. The first elution is performed with 200 ml of 5 to 6% ethyl ether in hexane, and the second elution with 200 ml of 15% ethyl ether in hexane. The K-D apparatus containing the eluates are connected to three ball Snyder columns and the solvents are evaporated as described in (5.1.2).

6.1.1 Eluate Composition - If the Florisil has been properly activated (cf. *"Official Methods of Analysis of the AOAC"*) and stored, and if the reagents are carefully prepared, the following eluate

compositions will be obtained when the pesticides are present. The first eluate (5-6% ethyl ether in hexane) will contain:

lindane	Perthane
BHC	heptachlor epoxide
Kelthane	methoxychlor
aldrin	toxaphene
heptachlor	Strobane
DDE	chlordane (γ & tech)
TDE	endosulfan I
DDT	

The second eluate (15% ethyl ether in hexane) will contain:

dieldrin	endosulfan II
endrin	lindane (possible trace of total)
	Kelthane (possible trace of total)

Standard pesticide mixtures should be used frequently to demonstrate the effectiveness of the Florisil to characterize the eluate composition and provide quantitative recovery. The concentrated extract may be analyzed directly by injecting suitable aliquots from the K-D ampoules into the gas chromatograph (8.). If the residues are high in total organics (as defined in 5.1.3) they may be further cleaned up and separated by thin layer chromatography prior to gas chromatographic analyses.

6.2 Thin Layer Chromatography (TLC)

The sample extract (5.1.3 or 5.2) or the Florisiled (6.1) sample extracts may be treated by TLC to separate pesticides and remove interfering substances.

6.2.1 Layer Preparation - Layers of silica gel 0.25 mm thick are

prepared on 200 x 200 mm glass plates. A homogeneous slurry is prepared by shaking 30 g of silica gel G in about 60 ml of water. With the aid of a variable thickness applicator, the slurry is immediately spread over five plates held on a mounting board. The layers are allowed to stand for five minutes. Then they are activated in an oven for 60 minutes at 110 C and stored in a desiccator for future use. Layers stored longer than one week should be reactivated before use.

6.2.2 Developing Solvent - The developing solvent, carbon tetrachloride, is added to the developing chamber to a depth of 10 mm. Two filter paper wicks, one on each side of the chamber, are placed so that one end contacts the solvent. After the lid is in place, the chamber is allowed to equilibrate for one hour. It is important that the chamber be protected from drafts and large temperature changes.

6.2.3 Sample Spotting - The sample extracts contained in K-D ampoules (6.1) or extracts retained (5.1.3, 5.2.1) and transferred to ampoules are carefully evaporated in a bath of warm water at 40 C with a fine draft of clean air to about 50-100 µl.* The internal wall of the ampoule is washed down with several 50 µl portions of ethyl ether and evaporated by the same technique to about 50 µl very carefully. The volume in the ampoule is adjusted to exactly 100 µl with benzene and a measured aliquot is spotted using a micro syringe. The entire sample may be spotted, if necessary, by

*Utmost care must be exercised to keep evaporation losses to a minimum. The use of "keeper", as discussed in 8.1, is not recommended here.

using several small applications, transferring the entire contents of the ampoule each time as thoroughly as possible with a micro syringe. Utmost care must be exercised in keeping the spot small (less than 1 cm diameter). A gentle stream of clean, dry air or nitrogen may be applied over the spot to facilitate close boundary evaporation, but this gas flow exposure should be kept to a minimum. With the aid of a spotting template up to nine spots, including samples and standards, may be spotted 20 mm apart and 10 mm from the bottom of the layer. Samples may be spotted in duplicate depending on the nature of corroborative evidence desired.

6.2.4 Standards - A mixture of standards containing 10-20 µg of each pesticide is spotted on each plate to confirm the separation of the respective pesticides by visual observation. Standards for recovery and instrumental measurement are spotted in the 20 to 100 nanogram range and handled just as a sample would be treated in the subsequent steps of the procedure.

6.2.5 Layer Development - The spotted layer is placed in the pre-equilibrated chamber (6.2.2) so that the bottom edge is in contact with the developing solvent and the lid is replaced. When the developing solvent reaches the upper reference line (10 cm), the layer is removed from the chamber and allowed to air dry at room temperature.

6.2.6 Visualization and Sectioning of Layer - After development, the portion of the layer containing the standards (10-20 µg) is sprayed evenly with a fairly heavy coat of Rhodamine B (0.1 mg/ml in ethanol). The sprayed area is allowed to thoroughly dry (about 5

minutes) and then is exposed to and viewed under short wave UV light. The pesticides show up as quenched areas (dark) on a fluorescent back ground. Mark the location of each pesticide.

The distance of travel for pesticides present in the unknown samples and recovery test standards will be, respectively, the same as those of the sprayed standards. From this information the vertical zone for each sample is divided into five horizontal sections. The sections are identified with Roman numerals as shown in Figure 1. Examples of respective Rf and Rr values for various pesticides are listed in Table 1.

6.2.7 Pesticide Removal From the TLC Plates - Using the spotting template as a ruler and with the aid of a sharp pointed object, the silica gel sections of interest are individually ruled off. With the aid of a mild vacuum, the silica gel, first from the periphery of the section and then from the center of the section, is drawn into a medicine dropper which is plugged at the tip with filtering grade glass wool (Figure 2). The pesticides adsorbed on this silica gel are eluted quantitatively into a 5 ml K-D ampoule or a 15 ml graduated centrifuge tube with successive small washes of ethyl ether-petroleum ether (1+1) to a total volume of 5 to 10 ml. The ampoule or tube is Tefion or glass stoppered and the contents are retained for the Determinative Steps (8).

7. Gas Liquid Chromatography

Reasonably positive identification of a pesticide is obtained by corroboration of the results using, at least, two different types of gas chromatographic columns. To achieve this, a relatively non-polar

packing and a semi-polar packing are employed. Packings and packed columns can be obtained from commercial sources or may be prepared in the laboratory.

7.1 Column Preparation

7.1.1 Non-Polar Packing - Dissolve 5 g of OV-17 in 225 ml of methylenechloride - chloroform (1+1) solution in a 500 ml beaker. Add slowly 95 g of Gas-Chrom Q (60-80 mesh) with constant stirring, under a stream of clean air in a hood. Care should be exercised to prevent crushing the Gas-Chrom Q. When the materials become only damp, place the contents of the beaker in a flat baking dish and spread evenly with occasional gentle agitation until no odor of chloroform or methylenechloride is detected. Dry in an oven at 120 C to allow the solvent to evaporate completely.

7.1.2 Semi-Polar Packing - Dissolve 5 g of QF-1 (FS-1265) and 3g of DC-200 in 225 ml of methylenechloride - chloroform (1+1) solution and add to 92g of Gas-Chrom Q (60-80 mesh) and proceed as in (7.1.1).

7.2 Column Material and Dimensions

The column may be constructed of either aluminum or borosilicate glass. In some cases highly heat-sensitive pesticides will chromatograph more reliably with a glass column.

The most useful length of column is about 6 ft. with a diameter of 1/4 in. O.D. to 1/8 in. O.D. depending on the detector employed, and the volume of a sample injection. Capture detectors of parallel plate design will perform well with either 1/4 in. or 1/8 in. columns, while the non-radioactive and concentric tube capture detectors operate best with a 1/8 in. column. A 1/4 in. column for the microcoulometric

detection system is recommended.

7.3 Packing the Column

The manner employed to pack the column is dependent on whether the column is glass (fixed shape) or aluminum.

7.3.1 Aluminum - Pour the respective packing material (7.1.1, 7.1.2) through a funnel connected by Teflon tubing to an aluminum tube which is plugged at the bottom with silanized glass wool. While pouring the material, tap the column continuously. A vibrator may be used to assist with the packing. (Caution: Vigorous vibration may crush the packing.) When the column is full plug the top with silanized glass wool.

7.3.2 Glass - Pour the respective packing material (7.1.1, 7.1.2) through a funnel connected by Teflon tubing to the glass column which is plugged at the exit with silanized glass wool. While pouring the material into the column use vacuum on the exit and agitate with a vibrator. After the column is full plug the end with silanized glass wool.

7.4 Conditioning Columns

The procedure recommended below is excellent for rapid conditioning of columns. However, alternate procedures may be employed.

Install the packed column in the gas chromatograph without connecting to the detector device. Heat the column to 270 C without gas flow for two hours. Allow the column to cool to 225 C and maintain this temperature for a minimum of 30 minutes (Caution: The column temperature must attain equilibrium to avoid bleed off of

liquid phase.) then adjust carrier gas to about 50 ml per minute for a 1/4 inch column and about 25 ml per minute for a 1/8 inch column. Aft one hour increase column temperature to 240 C and condition the colum for 24-48 hours. Cool the column down to operating temperatures 180-21 C and connect it to the detector system. Columns prepared and conditioned in the prescribed manner should yield good chromatograms without major losses of heptachlor or DDT with no further treatment. Even with the most advanced equipment the analyst is reminded that the system can be no better than the condition of the column. Injection of samples which have not been adequately prepared will shorten the life of the column and yield poor quality chromatograms.

8. Determinative Steps

In order to achieve the sensitivity described for the two forms of detection (MC and EC) described (4.2), a sample equivalent of approximately 1.0 liter must be measured by microcoulometry and 100 ml by electron capture. Further, the analyst must determine the optimum conditions to obtain the best results for compounds under study with the available equipment. Standard mixtures of interest should be chromatographed to determine the required separation with maximum resolution. Parameters such as gas flow, temperature, column length and diameter, as well as detector adjustment must be evaluated and altered as required to achieve the desired results.

8.1 Final Concentration

The sample extracts contained in a calibrated graduated centrifuge tube or a K-D receiver ampoule from the appropriate extraction or

purification step (5.1.2, 5.2, 6.1, or 6.2.7) is placed in a beaker of warm water (ca. 60 C). It is carefully evaporated with a fine stream of dried and filtered air to no less than 0.3 ml. The internal wall of the tube is washed down with hexane intermittently during this final concentration step and the volume is adjusted with hexane to an appropriate level - 0.3 ml to 0.5 ml for MC or 0.5 ml to 1.0 ml for EC.

If more exhaustive evaporation of the extract, < 0.3 ml, is required to achieve the necessary sensitivity for MC, the use of "keeper" is strongly recommended. Two milligrams of "keeper" is placed in the concentrated extract through syringe addition of 100 µl of 20 µg/µl Nujol in hexane. This "keeper" will not interfere with microcoulometric detection and will prevent major residue losses in this exhaustive evaporation step.

8.2 Sample Measurements

The volume of the sample extracts (8.1) is noted and suitable aliquots (20-100 µl for MC and 5-10 µl for EC) are analyzed by gas chromatography, employing non-polar and semi-polar columns for identification and quantitation. Standards are injected frequently, to insure optimum operating conditions. Gas chromatograms of several standard pesticides are shown in Figures 3 and 4. The elution order as well as elution ratios for various pesticides are provided in Tables 2 and 3, only as a guide. It is the responsibility of the analyst to develop his own identification keys to fit the chosen operating conditions of the instrument.

8.2.1 Calculations - The pesticide concentrations are determined as follows: Peak areas are calculated by disc integrator or planimeter

and compared to a standard calibration curve which is prepared daily by frequently obtaining peak areas of known quantities of the individual pesticide under identical conditions. Pesticide concentrations may also be determined by direct comparison to a single standard when the injection volume and peak area are very close to that of the sample. Calculation, from a standard calibration curve:

$$\text{nanograms/liter} = \frac{(Wi)\ \ (Vt)}{(Vi)\ \ (Vs)}$$

where

Wi = Weight of pesticide (ng) per injection

Vi = Volume of extract injected (µl)

Vt = Volume of total extract (µl)

Vs = Volume of water extracted (l)

Calculation from a single known standard peak area:

$$\text{nanograms/liter} = \frac{(A)\ \ (B)\ \ (Vt)}{(Vi)\ \ (Vs)}$$

$$A = \frac{\text{ng std}}{\text{std area}}$$

B = sample aliquot area

Vi = volume of extract injected (µl)

Vt = volume of total extract (µl)

Vs = volume of water extracted (l)

8.3 Reporting Results

Report results in nanograms per liter without correction for recovery data. The percent recoveries of known pesticides added to samples or to distilled water as well as the step in the

procedure where they were added should also be reported. Recoveries obtained at the 25 to 100 ng/l level vary with the individual pesticide but, should not fall below 50 percent for the entire procedure. Examples of overall recoveries obtained by a single laboratory are listed in Table 4.

If a sample is reported negative for a pesticide of interest, the analyst should also report a minimum detectable limit for that material. Sample response of less than two times the detector noise level (N) should be reported as negative. For sample response at two times the detector noise level, list the result as presumptive. Responses of greater than 2N should be quantified if possible. In cases of questionable identification the analyst should qualify the reported result to insure that subsequent misinterpretation will not occur.

TABLE 1

SOME Rf AND Rr VALUES OF PESTICIDES DEVELOPED WITH CCl_4 ON SILICA GEL G THIN LAYER PLATE

Pesticides & Metabolites	Rf Value	Rr Value	Section
Dieldrin	0.17	0.33	
Endrin	0.20	0.37	II
Heptachlor Epoxide	0.29	0.52	
Lindane	0.37	0.69	
DDD	0.54	1.00	III
γ-Chlordane	0.55	1.02	
Heptachlor	0.67	1.24	
DDT	0.68	1.26	IV
DDE	0.72	1.33	
Aldrin	0.73	1.35	

Rf = distance traveled by the compound divided by the distance traveled by the solvent front.

Rr = distance traveled by the compound divided by the distance traveled by standard p,p'-DDD.

TABLE 2

RETENTION TIMES FOR SEMI-POLAR COLUMN

Pesticide	Retention Time (Minutes)	Approximate Relative Retention[a]
BHC	2.0, 2.5, 3.4	0.47, 0.59, 0.80
Lindane	2.5	0.59
Heptachlor	3.4	0.80
Aldrin	4.2	1.0
Heptachlor epoxide	6.5	1.5
Kelthane	7.0	1.7
Endosulfan I	8.7	1.9
p,p'-DDE	8.8	2.0
Dieldrin	10.5	2.5
o,p-DDT	11.5	2.7
p,p'-DDD	11.8	2.8
Endrin	11.9, 19.2	2.8, 4.6
Endosulfan II	14.6	3.1
p,p'-DDT	15.9	3.5
Methyoxychlor	25.4	6.0

[a]Ratio of retention time to that of aldrin

Instrument Parameters

Column:

Aluminum - 6 feet long, 1/4 inch O.D.

Support - Gas-Chrom Q (60-80 mesh)

Liquid Phase - 5% QF-1, 3% DOW 200

Carrier:

Nitrogen, 100 ml/min.

Temperatures:

Injection - 250 C.

Column Oven - 185 C.

Detector - 205 C.

Detector:

Electron Capture -

Tritium, parallel plate

TABLE 3

RETENTION TIMES FOR NON-POLAR COLUMN

Pesticide	Retention Time (Minutes)	Approximate Relative Retention[a]
BHC	1.9, 2.6, 3.0	0.46, 0.63, 0.73
Lindane	2.6	0.63
Heptachlor	3.3	0.80
Aldrin	4.1	1.0
Heptachlor epoxide	5.8	1.4
Kelthane	5.8	1.4
Endosulfan I	7.6	1.9
p,p'-DDE	8.9	2.2
Dieldrin	9.4	2.3
Endrin	11.7	2.9
o,p-DDT	13.0	3.2
p,p'-DDD	13.4	3.3
Endosulfan II	14.0	3.4
p,p'-DDT	16.3	4.0
Methoxychlor	32.5	7.9

[a]Ratio of retention time to that of aldrin

Instrument Parameters

Column:

Aluminum - 6 feet long, 1/4 inch O.D.

Support - Gas-Chrom Q (60-80 mesh)

Liquid Phase - OV-17

Carrier:

Nitrogen, 100 ml/min.

Temperatures:

Injection - 250 C.

Column Oven - 205 C.

Detector - 235 C.

Detector:

Electron Capture -

Nickel-63, parallel plate

TABLE 4

OVERALL RECOVERIES FROM DOSED DISTILLED WATER BY A SINGLE LABORATORY[a]

Pesticide	Concentration (ng/l)	Percent[b] Recovery
Lindane	25	82
Heptachlor	25	75
Aldrin	25	75
Heptachlor Epoxide	25	53
DDE	25	71
DDT	100	82
Dieldrin	25	82
Endrin	50	82
Endosulfan	50	51
Methoxychlor	100	82

[a]Based on recoveries using the following steps in the procedure: extraction, initial concentration, thin-layer clean-up and final evaporation.

[b]Average of three determinations.

FIGURE 1

DIAGRAM OF DESIGNATION OF TLC SECTIONS IN THE CLEANUP AND SEPARATION ON SILICA GEL PLATES

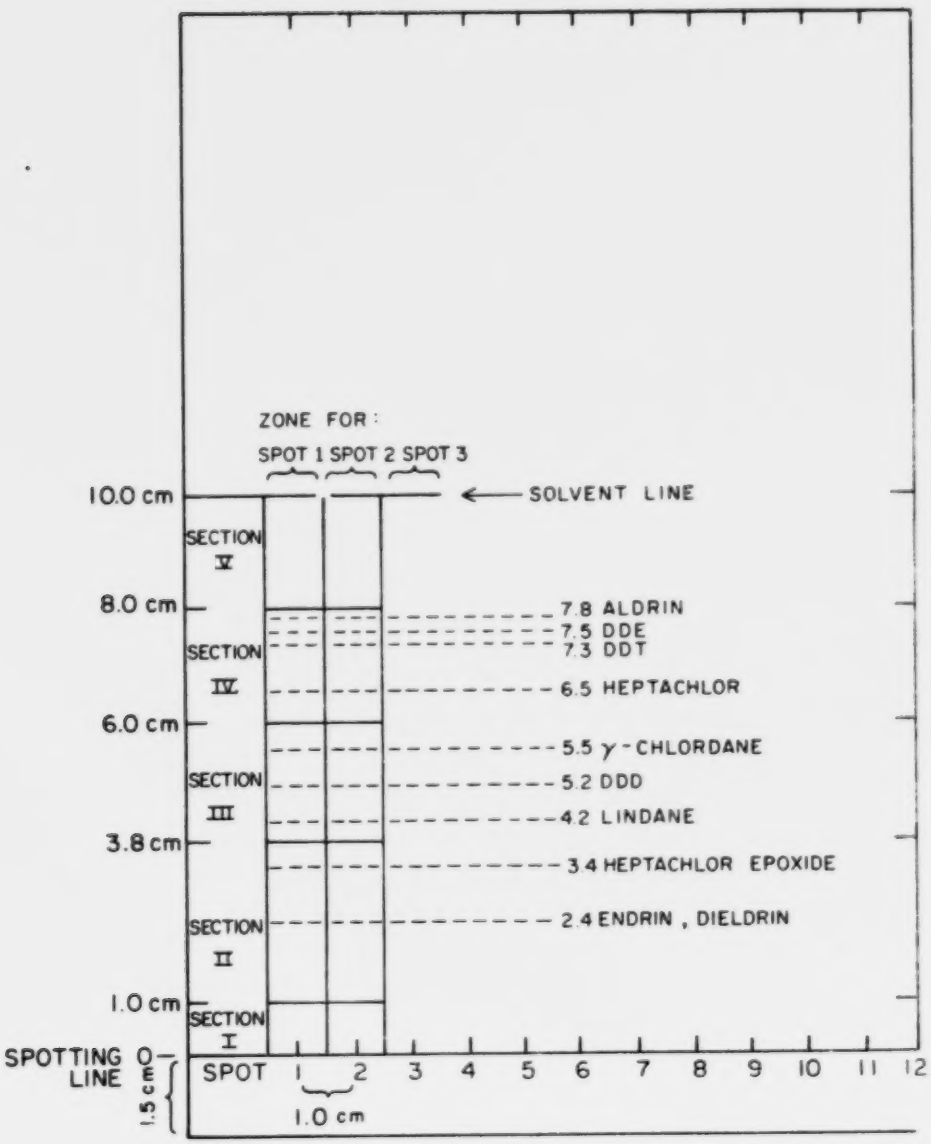


FIGURE 2

SILICA GEL COLLECTION ASSEMBLY

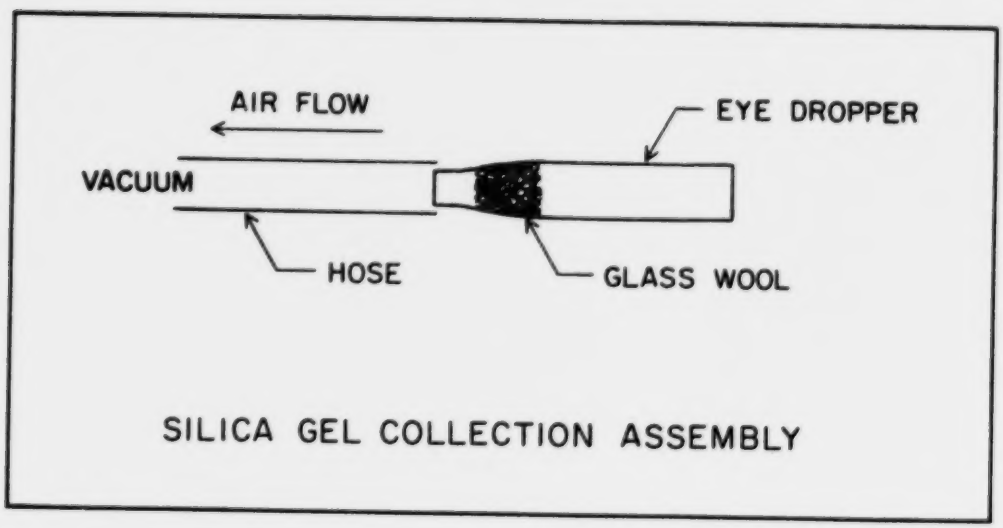


FIGURE 3
ELECTRON CAPTURE GAS CHROMATOGRAM OF PESTICIDE STANDARDS ON SEMI-POLAR COLUMN

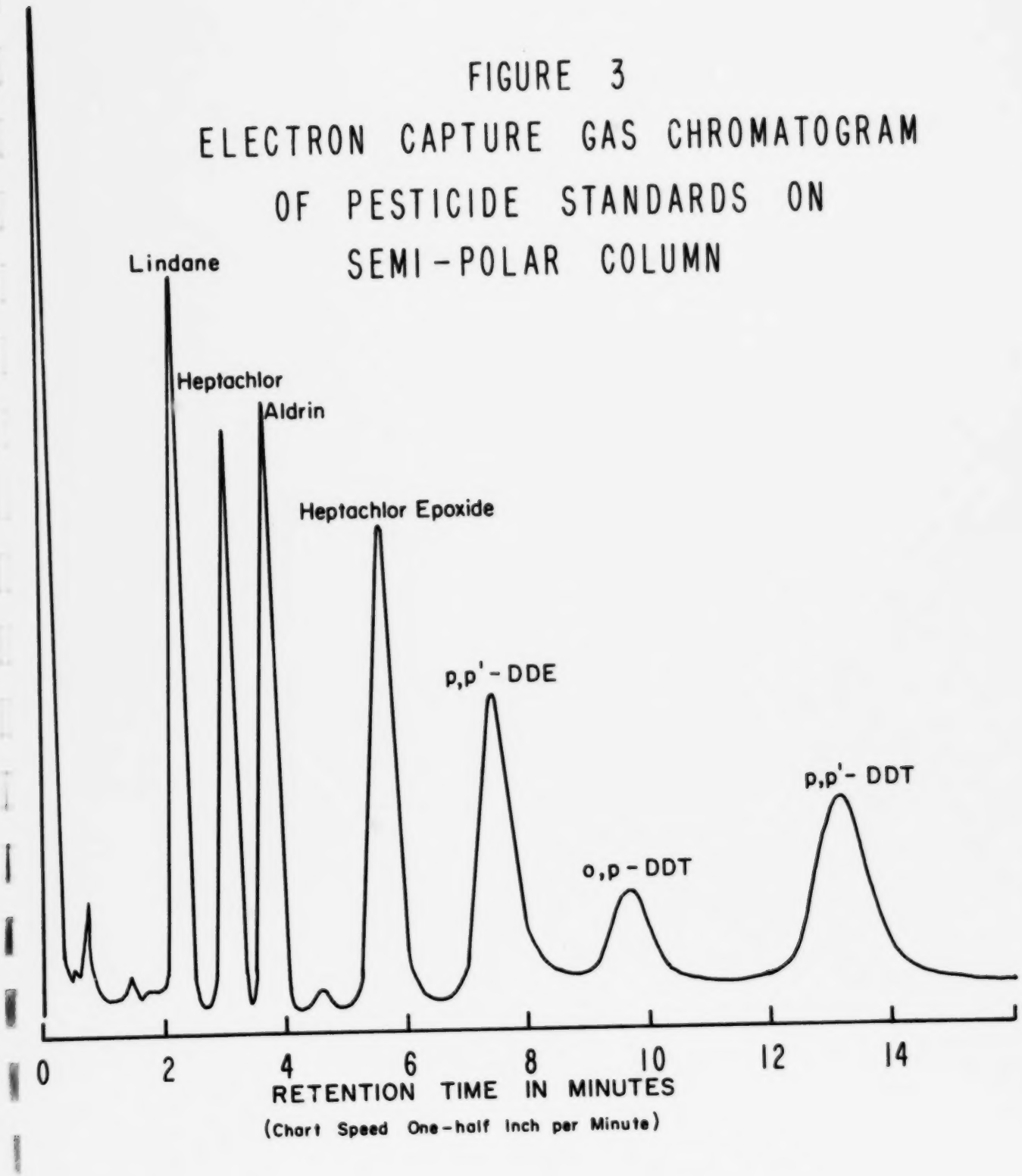
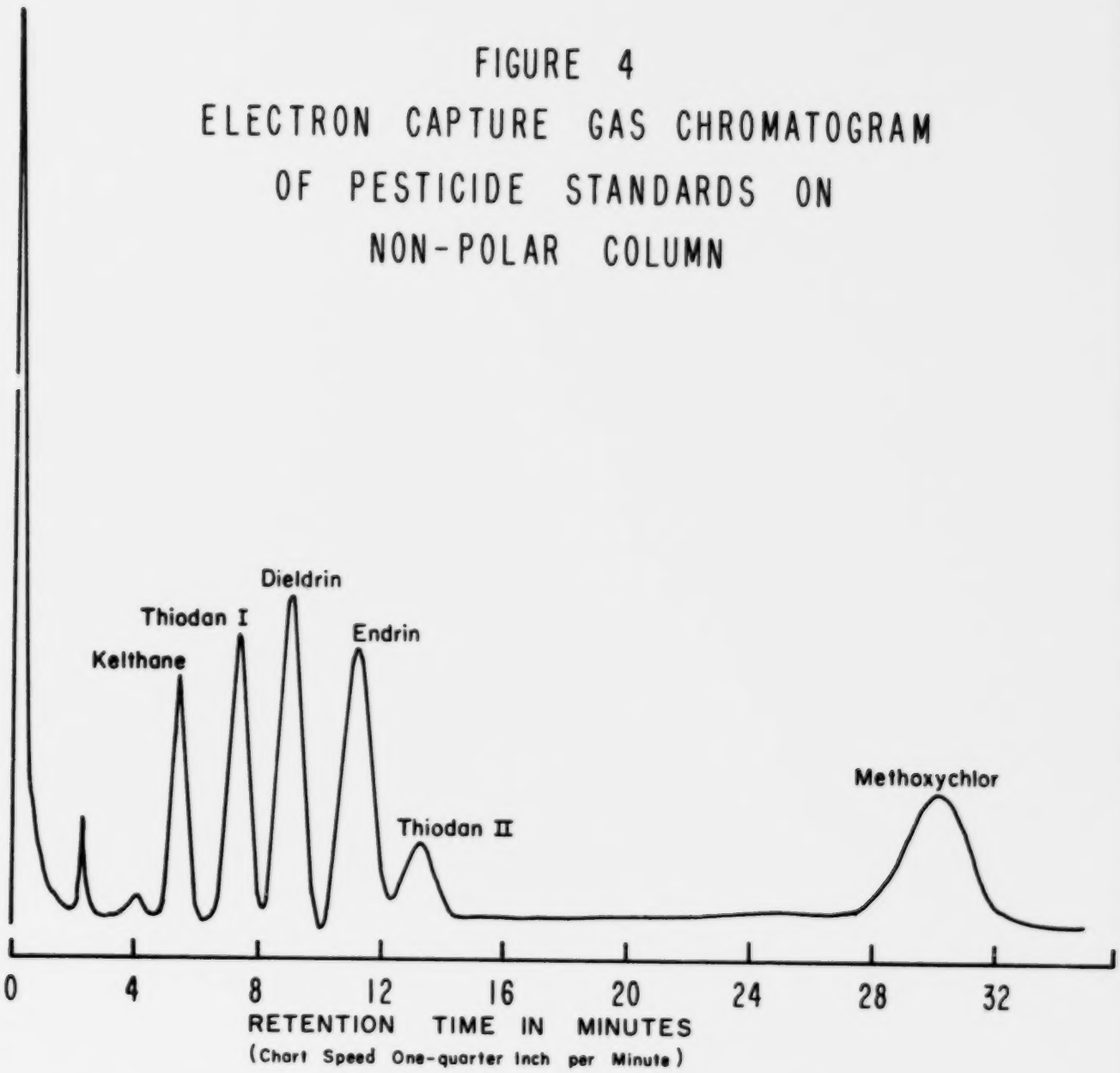


FIGURE 4
ELECTRON CAPTURE GAS CHROMATOGRAM OF PESTICIDE STANDARDS ON NON-POLAR COLUMN



REFERENCES

(1) McNair, H. M. and Bonelli, E. J., *"Basic Gas Chromatography"*, Varian Aerograph, Walnut Creek, California 94598, 1965.

(2) Heftmann, E. ed. *"Chromatography"*, Reinhold Publishing Corporation, New York 1961.

(3) Breidenbach, A. W., et al, *"The Identification and Measurement of Chlorinated Hyarocarbon Pesticides in Surface Waters"*, Publication WP-22, U.S. Department of the Interior, Federal Water Pollution Control Administration, Washington, D.C. 20242, 1966.

(4) Teasley, J. I. and Cox, W. S., *"Determination of Pesticides in Water by Microcoulometric Gas Chromatography after Liquid-Liquid Extraction"*, Journal American Water Works Association, 55, 1093 (1963).

(5) Stahl, E. ed., *"Thin-Layer Chromatography, A Laboratory Handbook"*, (Springer-Verlag, Berlin) Academic Press, Inc., Publishers, New York, 1962.

(6) *"Official Methods of Analysis of the Association of Official Agricultural Chemists"*, page 382, 10th Edition, Association of Official Agricultural Chemists, Washington, D.C. 20044, 1965.

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